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Parallel and Automated Formation of Lipid Bilayers on Microstructured Chips for Ion Channel and Nanopore Recordings

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Bilayer recording is a well-established technique for in-depth studies of biophysical properties of ion channels and is particularly suited for functional studies on proteins residing in intracellular membranes. Moreover, this technique supports a host of powerful emerging analytical techniques using biological nanopores as molecular sensors. Despite its proven value, bilayer recording can be frustrating due to the capricious nature of lipid bilayers, which have to be formed manually one by one and which often lack stability. We here show a new approach and device, which speeds up the entire process by the rapid and simultaneous formation of 16, highly stable micrometer-sized bilayers using Micro-Electrode-Cavity-Array (MECA)-Chips. A study will be presented showing that the MECA supports high-resolution polymer sizing with a single biological nanopore in a parallel format (Fig.1). Additionally, data on a variety of channel proteins recorded from proteoliposomes will be shown. using a surface containing micron-sized apertures in glass substrates, the fusion of vesicles on the surface becomes an attractive method for electrophysiology and then to reconstitute membrane proteins into the lipid bilayer.

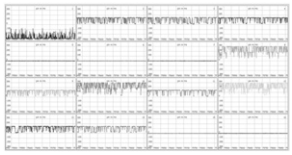

Fig. 1. MonoPEG-28-mediated blockages of HL-nanopore(s) on a MECA chip.

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Using Biophysics Tools to Probe Permeability of Ions in Porous Manganese Oxide

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Measurements of ion current and reversal potential at different salts have been used to get information on the effective opening, surface characteristics and ionic selectivity of biological channels and solid-state nanopores. For example a non-zero transmembrane potential recorded when a pore is in contact with an electrolyte concentration gradient is a quantitative measure of the pore's preference to an ion of a given charge and/or size. We applied these tools to study ionic properties of nano-voids present in manganese oxide. Mangenese oxide is used in many applications, e.g. batteries, however ionic transport through this material is not well-understood. Single polymer pores with opening diameters of ~100 nm were used as a template to electrochemically deposit wires of manganese oxide. The material assumes the shape of the pore thus the recorded ion current probes properties of the deposited manganese oxide and not the polymer template itself. Measurements of current-voltage curves at different electrolyte configurations revealed a negative surface charge of the manganese oxide nanovoids and sub<5 nm diameter of the voids' opening. The average opening diameter of the voids were found based on observation of ion current saturation at low salt concentrations, and a calculation of the Debye length. Conductance of the manganese oxide nanovoids in lithium, sodium and potassium salts does not follow the behavior of bulk solutions, which indicates that the voids are sufficiently narrow to differentiate between different monovalant ions.

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Voltage-Gated Synthetic Pores for Controlled Transport of Ions and Neutral Molecules

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Synthetic nanopores similar to biological channels are designed to allow for controlled transport of ions and molecules. We have developed a synthetic mimic of a voltage-gated channel equipped with an electromechanical gate that restricts or permits the transport as a function of external voltage. The gate is built from single-stranded DNA molecules attached to the small opening of an asymmetric, conical shaped nanopore. Voltage-dependent conformations of the ssDNA lead to voltage-dependent opening of the pore. In addition, the DNA conformation was found to be dependent on the ionic strength of the electrolyte allowing one to achieve different levels of control as a function of KCl concentration. Since changes in the DNA conformation occur inside a very restricted volume of a nanopore, hysteresis effects were observed, which could become the basis for construction of an ionic memristor. The memory and hysteresis effects are most pronounced in pores with the effective diameter below 5 nm. Voltage-gated synthetic pores were also shown to exhibit an on/off transition as a function of pH and the sequence of DNA molecules attached to the pore walls. Voltage and pH dependent pore opening was confirmed by spectroscopic studies of neutral dyes through membranes containing $10 \perp 5$ voltage-gated synthetic pores per $cm \perp 2$. This voltage-controlled system could find application in building separation membranes which would permit transport of differently sized species as regulated by the controlled pore opening.

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Supported Lipid Bilayer Nanopore Protein Gated All Semiconducting Nanotube Network Devices

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We demonstrate an integrated system in which all-semiconducting nanotube network transistors are coated directly with lipid bilayers which contain transmembrane ion channel proteins gramicidin A (gA) and α-Hemolysin (α-HL). Dynamic opening and closing of the pores is observed through measurement of the current from the nanotube network, through the nanopores, and into solution. The pores investigated pass either only cations (gA) or both anions and cations (a-HL), allowing a study of the effect of both species on the threshold voltage and mobility of the nanotube network. Blocking of the ion channel currents is demonstrated to occur with PEG, indicating potential applications in nanopore based sequencing technologies. The all-semiconducting nanotube network devices are compatible with low cost printed electronics, opening a window for massively parallel manufacturing of nanotechnology for a variety of applications in electrophysiology and biosensors.

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Precise Control of Solid-State Nanopore Device Properties for Single-Molecule Detection Applications

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Solid-state nanopores are an emerging technology for detection and analysis at the single-molecule level. Here, we discuss fabrication and characterization of nanopores and nanopore arrays using Helium Ion Milling. We investigate nanopore dimensions using atomic force microscopy and transmission electron microscopy. We demonstrate that nanopore diameter and local membrane thickness are controlled precisely through ion beam dose, achieving pore diameters below 3 nm and device thickness below 5 nm. The technique is high-throughput and lithographic patterning can be used to form arrays of arbitrary size. The resulting devices are compatible with both electrokinetic and optical detection schemes for biodetection applications.

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Probing Physical and Mechanical Properties of Particles in the Resistive-Pulse Technique

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The resistive-pulse technique is a powerful approach to detect single molecules and particles. A single particle passing through a pore can be observed as a transient drop of the transmembrane current. Our research focuses on resistive-pulse sensing experiments performed with track-etched polymer pores characterized by an undulating diameter along the pore length. The resistive pulses generated by spherical beads passing through these pores have a repeatable pattern of large variations corresponding to these diameter changes. We show that this pattern of variations enables the unambiguous resolution of multiple particles simultaneously in the pore, the detection of transient sticking of particles within the pore, and confirmation whether any individual particle completely translocates the pore. This pattern of variations was also found to be independent of the particle size. Pores with undulating diameter can also differentiate between particles of different shape but similar volume as demonstrated by our experiments with rod-shaped particles. This is due to particle interaction with the internal structure of the pore in a way that is specific to their size and shape. It is important to mention that distinguishing between various shapes is not possible with the classical resistive-pulse technique which is based exclusively on the detection of particle volume. We have also performed resistive-pulse experiments with hydrogel particles which revealed their ability to squish and expel solvent during translocation. Pores with undulating pore diameter can therefore be also applied to probe mechanical properties of translocating particles.